AGE OF ANTARTIC FISH : VALIDATION OF THE TIMING OF ANNULI FORMATION IN OTOLITHS AND SCALES

by

A.W. NORTH (1)

ABSTRACT. - Growth zones in the otoliths and scales of Antarctic fish were investigated to determine during which period of the year they were formed. In otoliths the opaque zones were formed during summer and autumn, and the hyaline zones during the winter, spring and early summer. In scales, the zone of widely spaced sclerites (circuli) was formed during summer and autumn, and the zone of closely spaced sclerites in winter. The hypothesis that an adjacent opaque and hyaline zone in otoliths, and an adjacent wide and closely spaced sclerite zone in scales, is an annulus which represents one year of life was supported by this study. It was concluded that counting such annuli is a valid technique to age Antarctic fish.

RÉSUMÉ. - Les zones de croissance visibles dans les otolithes et sur les écailles de poissons de l'Antarctique ont été étudiées afin de déterminer la période de l'année durant laquelle elles se forment. Dans les otolithes, les zones opaques se forment pendant l'été et l'automne et les zones hyalines pendant l'hiver, le printemps et le début de l'été. Dans les écailles, les zones de sclérites (circuli) largement espacées sont déposées en été et en automne et les zones de sclérites resserrées en hiver. Cette étude soutient l'hypothèse que, dans les otolithes, deux zones adjacentes l'une opaque l'autre hyaline et, dans les écailles, deux zones adjacentes l'une avec des sclérites espacées et l'autre avec des sclérites resserrées, sont un annulus qui représente une année de vie. Compter de tels annuli apparaît donc comme une méthode valable pour déterminer l'âge de poissons de l'Antarctique.

Key-words : PSW Antarctica, Otoliths, Scales, South Georgia, Age determination.

Antarctic fish populations have been exploited by Russian and Polish fishing fleets during the last ten years (Kock *et al.*, 1985 ; Kock, 1986). The yield to the fishery has fallen over that period. The fish stocks and the average age of the fish at South Georgia have declined. These stocks are now protected by conservation measures under the international Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) which has proposed that the status of the stocks should be monitored (CCAMLR, 1986). It will be necessary to determine both the age and size structures of the fish populations to predict the potential yield to both the fisheries and natural predators.

Age of fish is usually estimated by the interpretation of patterns in the structure of hard tissues such as otoliths (sagitta), scales, vertebrae and other bones. The first information about the age of Antarctic fish resulted from an analysis of samples of *Notothenia rossii* from South Georgia by Olsen (1954). Olsen used a combination of the pattern of the sclerites in the scales and length-frequency plots to deduce age. To age three species of Channichthyids which lack scales, Olsen (1955) used annuli within otoliths combined with length frequency analysis. Since then it has been proved possible to age many species of Antarctic fish from scales

---

(1) British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 OET, UK.

Cybium 1988, 12 (2) : 107 - 114.

or otoliths (Everson, 1970, 1981 ; Hureau, 1970 ; Scherbich, 1975 ; White and North, 1979 ; North *et al.*, 1980). Other tissues have also been used for age determination, including pectoral girdle bones (Tomo and Cordero-Funes, 1972), vertebrae (Kochkin, 1980) and fin-rays (Freytag, 1980 ; Gubsch, 1980).

Previous studies have assumed that summer growth, is represented in otoliths by the opaque zone and in scales by the region of widely spaced sclerites, whereas winter growth is represented by the hyaline zone in otoliths and the closely spaced sclerite zone in scales (Everson, 1981). This assumption has been shown to be true for temperate fish (Williams and Bedford, 1974). In Antarctic fish the agreement between estimates of age from polymodal length frequency distributions and those from examination of otoliths and scales support the assumption that each pair of zones represents one year, though few studies have determined when the growth zones are formed or demonstrated that they are annual.

The age of *Pagothenia bernacchii* caught off Terre Adélie was investigated by Hureau (1964). Hureau stated that the zones in the otoliths were annual, with the opaque zone forming during November to February and the hyaline zone in March to July. The timing of annulus formation in the scales of *Notothenia rossii* from South Georgia was investigated by Scherbich (1975). Scherbich found that, in July, 86.6 % of the fish had a complete annulus on the margin of the scales. She used the first of July as the "annual boundary line" (birthday) to determine the age of the fish. The present study has investigated the outermost structures of otoliths and scales in order to determine when the summer and winter zones were formed.

MATERIAL AND METHODS

Fish were captured by using an Agassiz trawl towed along the seabed, or by SCUBA diver using a hand net. Collections were made in nearshore waters, mostly at Cumberland East Bay, South Georgia but also at Factory Cove, Signy Island, South Orkney Islands. The species of fish captured included, *Notothenia angustifrons, Notothenia neglecta, Notothenia rossii, Nototheniops nudifrons, Nototheniops larseni, Trematomus newnesi*, and *Harpagifer georgianus* (using the nomenclature of Fischer and Hureau, 1985).

Otoliths, and scales when present, were removed from fresh specimens, rinsed in freshwater, and stored dry in labelled paper envelopes or glass vials. Otoliths were cleared with cedar-wood oil and examined whole at x16 to x60 magnification using a binocular microscope and a high power light source (Volpi intralux 6000 with intense focus lens). Both reflected and polarised transmitted lights were used alternately to view each specimen. Dark field illumination was obtained when using transmitted light by a polarising filter both beneath the stage and the objective lens of the microscope. The filters were aligned 90 degrees out of phase to each other. This method of illumination made the internal structure of the otolith easier to discern than normal bright field illumination. A proportion of otoliths (7 %) were too opaque to be viewed by this method. They were cracked, charred and then viewed using reflected light (North *et al.*, 1980). Scales were viewed dry using transmitted light.

Nomenclature of the zones forming the annuli and the interpretation of the structures observed follows that of Everson (1981) :

"*Annulus* : An annulus is the combination of each opaque and hyaline zone proceeding away from the nucleus. The first annulus is a special case in that it consists of the nucleus plus the first true hyaline zone.

Opaque zone : Zone consisting of material which obstructs the passage of light

(usually formed during the summer or fast growing period).
Hyaline zone : Zone consisting of material which permits the passage of light (usually formed during the winter or slow growing period)."

Pseudo-hyaline edge : The outermost edge of otoliths immersed in cedar wood oil and viewed by transmitted light usually appears translucent. Even an opaque zone which is not yet complete appears to have a peripheral hyaline edge. Therefore this peripheral edge must be the growing part otolith which has not yet differentiated into a hyaline or opaque zone. I have called this outermost edge the pseudo-hyaline edge. The radius of the pseudo-hyaline edge represents an unknown period of time. An indication of this interval is the radius of the pseudo-hyaline edge as a proportion of the total radius of the otolith. To estimate this period it was assumed that the rate of deposition is about equal for the pseudo-hyaline edge and the fully differentiated part of the otolith. From this assumption it follows that the time represented by the pseudo-hyaline edge as a proportion of the total time taken to form the otolith is analogous to the radius of the pseudo-hyaline edge as a proportion of the total otolith radius. Based on this assumption the following equation was used for each individual observation to estimate the time represented by the pseudo-hyaline edge :

$$\frac{\begin{array}{c}\text{Radius of the pseudo-hyaline edge along}\\ \text{the otolith radius}\end{array} \quad \text{X} \quad \begin{array}{c}\text{Months represented by the total otolith}\\ \text{radius} + 2^{*}\end{array}}{\text{Total otolith radius}}$$

The individual estimates were then pooled to give an overall estimate of the time represented by the pseudo-hyaline edge. This was found to be : 0.70 to 1.18 months ($\bar{x}$ = 0.94, s.d. 0.24, min. 0.30, max. 2.10, n = 44). Therefore an error of about one month is likely in observations of when the outermost zone in otoliths was formed. This difference, or error, between the date of sampling otoliths and the time when the edge of the last zone was formed is about 8 % of a year.

For effective statistical analysis it was necessary to pool observations of otolith zones for several months because only a small number of samples were obtained each month. This further reduces the precision in determining when the zones were formed. These reductions of the precision may result in a combined error of about two months.

The criteria followed to make an objective estimate of the type of peripheral zone observed in otoliths and scales is given in Table I. The chi-square test was used to determine whether the observed distributions (Figs 1, 2 ; Tables II, III) were significantly different from a uniform distribution (Snedecor and Cochran 1980).

RESULTS

OTOLITHS

In otoliths the type of outer zone observed is shown in Fig. 1, Table II. The data includes several species (n = number of specimens) ; *Notothenia angustifrons*

---

* Note 2 months was added to compensate for the unknown period of time represented by the pseudo-hyaline edge.

Table I : Description of the outermost zone in otoliths and scales *.

Age-stage	Otoliths	Scales	Width of zone
1	opaque	widely spaced sclerites	narrow
2	opaque	widely spaced sclerites	well developed
3	opaque	widely spaced sclerites	wide, almost equal to width of previous similar zone
4	hyaline	closely spaced sclerites	narrow
5	hyaline	closely spaced sclerites	well developed

* Note this excludes the extreme outermost edge of otoliths which nearly always appears hyaline in transmitted light.

Table II : Percentage (and numbers) within each age stage from observations of the outer zone of otoliths ; data pooled over two month periods.

Stage	Months				
	12 + 1	3 + 4	5 + 6	7 + 8	9 + 10
1	36 (9)	0 (0)	0 (0)	0 (0)	5 (2)
2	16 (4)	63 (10)	0 (0)	8 (2)	8 (3)
3	20 (5)	19 (3)	90 (9)	4 (1)	5 (2)
4	4 (1)	19 (3)	0 (0)	67 (16)	23 (9)
5	24 (6)	0 (0)	10 (1)	21 (5)	60 (24)

Table III : Percentage (and numbers) within each age stage from observations of the outer zone of scales; data pooled over three month periods.

Stage	Months	
	1 + 3 + 4	7 + 8 + 9
1	0 (0)	0 (0)
2	50 (17)	7 (3)
3	29 (10)	9 (4)
4	12 (4)	24 (11)
5	9 (3)	61 (28)

(39), *Notothenia neglecta* (2), *Notothenia rossii* (8), *Nototheniops nudifrons* (47), *Nototheniops larseni* (1), *Trematomus newnesi* (4), and *Harpagifer georgianus* (19). A chi-square test of the number in each-stage zone throughout the year indicated that it was not a uniform distribution (X^2_{16} $p<<0.005$). The results (Fig. 1) show that in the majority of otoliths there was a progressive sequence of zones throughout the year. There is either a well-developed hyaline zone or a narrow opaque zone during

December and early January (stage V or I), a well developed opaque zone during March and April (stage II), a wide opaque zone in May and June (stage III) (almost equal in width to the previous complete opaque zone), a narrow hyaline zone in July and August (stage IV), and a well developped hyaline zone in September and October (stage V).

Particular stages in the development of the annulus were only found during certain periods of the year. An outer opaque zone was found from January to June during the austral summer and autumn ; whereas an outer hyaline zone was found during July to January, in the southern winter, spring and early summer. This demonstrates that the opaque and hyaline zones are formed at different periods of the year and that an annulus consisting of an adjacent opaque and hyaline zone is formed each year. A very narrow opaque zone was observed on the outer edge of some otoliths, from October (n = 3) and early January (n = 2) (no observations from November). The opaque zone was therefore initiated during October to January (in spring and early summer). The intiation of the hyaline zone was difficult to identify due to the pseudo-hyaline edge of the otolith. Observations classified as a narrow hyaline zone during April and May (Fig. 1) were probably errors resulting from difficulties in discriminating between the hyaline zone and the pseudo-hyaline zone. A wide opaque zone was observed in the majority of otoliths from May and June, but during July and August most of the otoliths sampled had a narrow hyaline zone. Therefore the beginning of the hyaline zone was during the winter.

SCALES

The type of zone found on the outermost edge of scales for three month periods is shown in Fig. 2 and Table III. Data from several species were combined (number of specimens) : *Notothenia angustifrons* (65), *Notothenia neglecta* (2), *Notothenia rossii* (11) and *Nototheniops nudifrons* (45). From the observations, the most common age-stage zones in scales were a zone of widely spaced sclerites during January to April (stage II), and a well developed zone of closely spaced sclerites

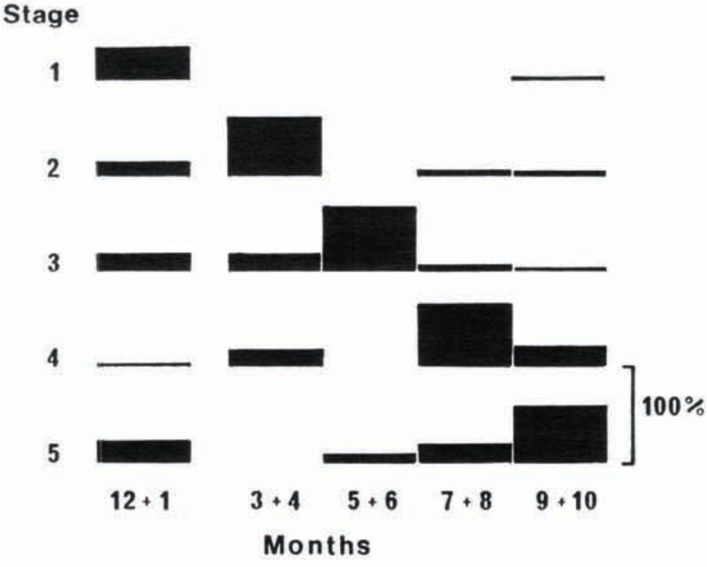

Fig. 1 : Age-stage zones observed on the outer edge of otoliths as a percentage of the number sampled. Data pooled for two month periods.

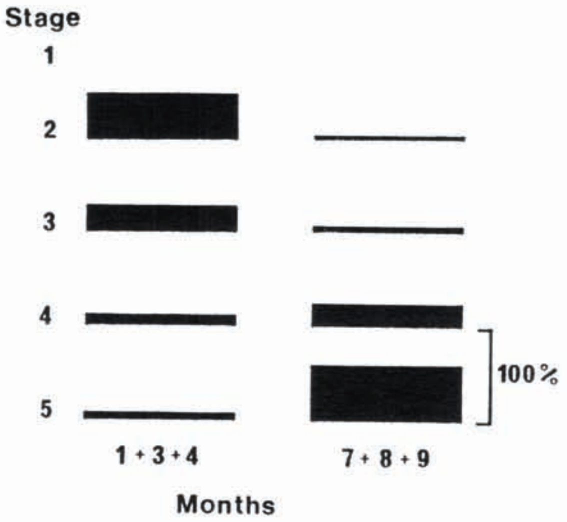

Fig. 2 : Age-stage zones observed on the outer edge of scales as a percentage of the number sampled. Data pooled over three month periods.

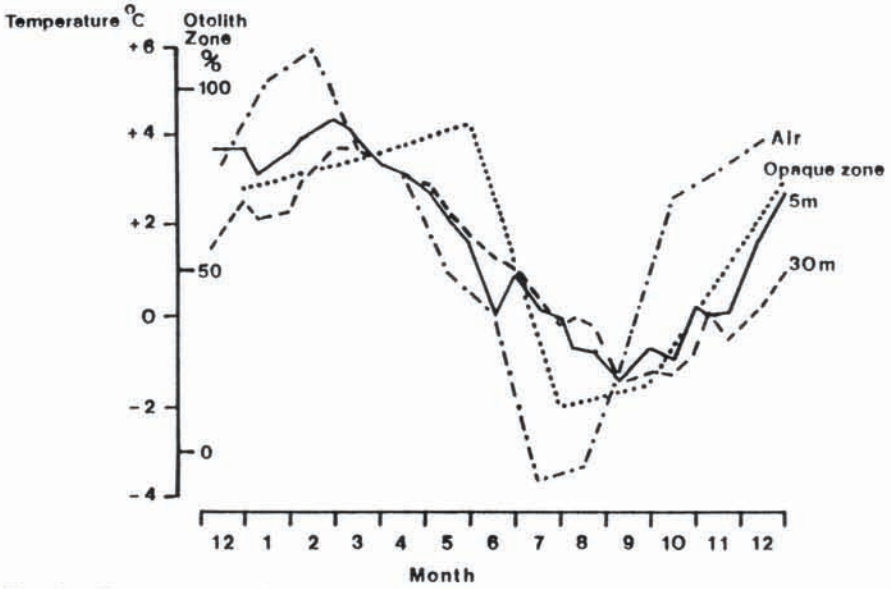

Fig. 3 : Temperatures at Cumberland East Bay, South Georgia during 1980 ; _._._._ air at Grytviken (monthly mean), ______ sea at 5m depth in King Edward Cove, and - - - - sea at 30m depth (in the bay outside the entrance to the cove) (Limbert, 1980, 1981a and 1981b; Forster, 1980); and the .... percentage of otoliths with an opaque zone on the edge (Table II).

during July to September (stage V). A chi-square test confirmed that the age-stage zones were not uniformly distributed throughout the year (X^{2}_{4} $p \ll 0.005$). In scales the peripheral zone of widely spaced sclerites was found in specimens collected during the summer and autumn, and of closely spaced sclerites during the winter.

DISCUSSION

The pseudo-hyaline edge in otoliths was estimated to represent about one month of growth. This reduces the precision in determining when different types of zones were formed in otoliths. That is, in otoliths the differentiated opaque or hyaline zone observed was formed about one month before the otolith was sampled from the fish. The opaque zone in otoliths was initiated in October to January, and developed during November to June. It therefore represents a summer and autumn zone. The hyaline zone was initiated in July and August and was found on the periphery of the majority of otoliths from August to December. It is therefore a winter and spring zone. Fish of the same year class at certain times of the year may have a different type of peripheral zone on their otoliths. In December (Fig. 1) the peripheral zone in the otoliths of some individual fish was hyaline, but in others there was the beginning of an opaque zone. If these fish are of the same year class and they were sample during December they could be assigned different ages. To avoid this problem only complete annuli consisting of an adjacent opaque and hyaline zone should be counted as one year. Hureau (1964) has suggested that temperature may affect the type of zone formed in otoliths, and in this study the greatest percentage of otoliths having an outer opaque zone were found during the periods of the year when sea temperatures were highest (Fig. 3). However temperature and season affect many aspects of the condition and behaviour of Antarctic fish. Targett (pers. comm.) found that temperature had a profound effect on the feeding rate of *Harpagifer antarcticus*. Burchett (1983) found that the mean relative liver size in *Notothenia rossii* at South Georgia was high from March to August (autumn to early winter) and low from September to February (late winter to summer).

Clearly a thorough investigation of the development of hard tissues, in relation to both environmental (temperature, photoperiod, etc.) and endogenous (reproductive and nutritional status, hormone cycles, etc.) variables would be of considerable value.

This study validates the assumption that two zones are formed in both otoliths and scales during one year, which together constitute one annulus. The use of annuli in scales and otoliths to age Antarctic fish is therefore a valid technique if the interpretation and nomenclature suggested by Everson (1981) are used.

Acknowledgements. - I wish to thank Robert Forster for assistance with obtaining some of the specimens, Pete Rothery for suggesting the use of the chi-square test, Barry Heywood, Martin White and Roger Coggan for improvements of the manuscript.

REFERENCES

CCAMLR., 1986. - *Report of the fifth meeting of the Scientific Committee (Hobart, Australia, 8-15 September 1986).* SC-CAMLR V, Hobart, Australia, 271 pp.

BURCHETT M.S., 1983. - Age and growth of the Antarctic fish *Notothenia rossii* from South Georgia. *Bull. Br. Antarct. Surv.*, 60 : 45-61.

EVERSON I., 1970. - The population dynamics and energy budget of *Notothenia neglecta* Nybelin at Signy Island, South Orkney Islands. *Bull. Br. Antarct. Surv.*, 23 : 25-50.

EVERSON I., 1981. - Antarctic fish age determination methods. *BIOMASS Handbook*, 8, 24 pp.

FISCHER W. & J.-C. HUREAU, 1985. - *FAO species identification sheets for fishery purposes. Southern Ocean (Fishing areas 48, 58 and 88) (CCAMLR Convention Area).* Prepared and published with the support of the Commission for the Conservation of Antarctic Marine Living Resources. Rome, FAO, Vol. 2, 233-470.

FORSTER R.T., 1980. - Marine Assistants Report, Grytviken, December 1979 - January 1981. *Br. Antarct. Surv. Rep.*, N1/1980/M 27 pp., (unpublished).

FREYTAG G., 1980. - Length, Age and Growth of *Notothenia rossii marmorata* Fischer 1885 in the West Antarctic waters. *Arch. FischWiss.*, 30 (1) : 39-66.

GUBSCH G., 1980. - Untersuchungen zur Altersbestimmung und zum Wachtstum beim Eisfish *Chaenocephalus aceratus* Lonnberg. *Fischerei-Forschung Wissen-schaftliche Schriftenreihe*, 18 (1) : 7-10.

HUREAU J.-C., 1964. - Contribution à la connaissance de *Trematomus bernacchii* Boulenger. *In* : Biologie antarctique, comptes rendus du premier Symposium de biologie antarctique, Paris, september 1962. Paris, Hermann, 481-487.

HUREAU J.-C., 1970. - Biologie comparée de quelques poissons antarctiques (Nototheniidae). *Bull. Inst. Océanogr. Monaco*, 68 (1391) : 244 pp.

KOCHKIN P.N., 1980. - How to prepare fish vertebrae for age determination. *J. Ichthyol.*, 20 (6) : 153-157.

KOCK K-H., 1986. - The state of exploited fish stocks in the Scotia Arc Region during SIBEX (1983-1985). *Arch. FischWiss.*, 37 (1) : 129-186.

KOCK K-H., DUHAMEL G. & J.-C. HUREAU, 1985. - Biology and status of exploited Antarctic fish stocks : a review. *Biomass Scient., Ser.* 6 : 143 pp.

LIMBERT D., 1980. - Monthly temperature summary for British Antarctic Survey Stations. *Polar Record*, 20 (125) : 175.

LIMBERT D., 1981a. - Monthly temperature summary for British Antarctic Survey Stations. *Polar Record*, 20 (127) : 378.

LIMBERT D., 1981b. - Monthly temperature summary for British Antarctic Survey Stations. *Polar Record*, 20 (128) : 455.

NORTH A.W., WHITE M.G. & M.S. BURCHETT, 1980. - Age determination of Antarctic fish. *Cybium, 8* : 7-11.

OLSEN S., 1954. - South Georgia cod (*Notothenia rossii marmorata* Fischer). *Norsk. Hfalvfangsttid.*, 7 (43) : 373-382.

OLSEN S., 1955. - A contribution to the systematics and biology of the Chaenichthyid fishes from South Georgia. *Nytt. Mag. Zool.*, 3 (1) : 79-93.

SHCHERBICH L.V., 1975. - Method of determining age and onset of sexual maturity in the marbled Antarctic cod *Notothenia rossii marmorata*. *J. Ichthyol.*, 15 (1) : 82-87.

SNEDECOR G.W. & W.G. COCHRAN, 1980. - *Statistical Methods*. (Seventh Ed.). The Iowa State University Press, USA. 507 pp. (Chapter 11.6, page 200).

TOMO A.P. & J.R. CORDERO-FUNES, 1972. - Edad y crecimento en largo del *Harpagifer bispinis antarcticus*. *Contrib. Inst. Antart. Argent.*, 96 : 1-19.

WHITE M.G. & A.W. NORTH, 1979. - Offshore Biological Programme : Ichthyological Data. *Br. Antarct. Surv. Data*, 2 : 25 pp.

WILLIAMS T. & B.C. BEDFORD, 1974. The use of otoliths for age determination. *In* : Ageing of fish. Proc. Intern. Symp. organized by the Europ. Fish. Adv. Comm. FAO, Fisheries Society of the British Isles and the Freshwater Biological Association, at The University of Reading (Bagenal T.B., ed.) : 114-123.

Reçu le 21.07.1987

Accepté pour publication le 5.09.1987.